

Supplemental Information

Inventory of Supplemental Information

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Figure S1

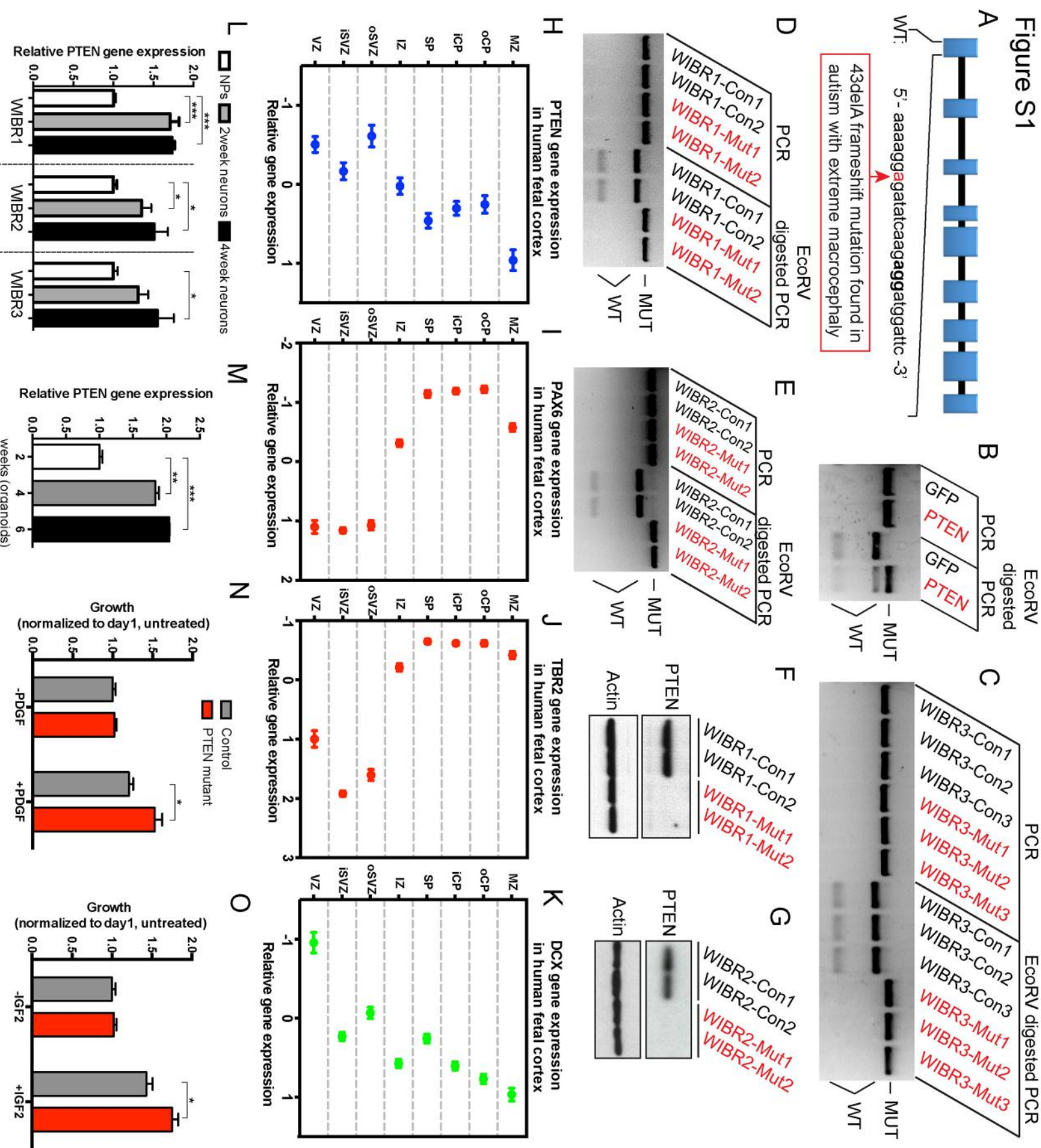


Figure S1. CRISPR/Cas9-mediated deletion of PTEN in hESCs, and PTEN expression analysis. Related to Figure 1.

A) Schematic overview of the human PTEN locus showing the heterozygous 43delA loss-of-function mutation identified in autism patient with macrocephaly (Marchese et al., 2014).

B) Gel images showing EcoRV digest results from HEK293 cells transfected with PTEN CRISPR/Cas9 and mock control with GFP plasmid. Indels generated in the target regions may lead to disruption of the EcoRV site.

C-E) Gel images showing disruption of the EcoRV site in PTEN mutant clones generated from WIBR3 (C), WIBR1 (D) and WIBR2 (E) hESCs.

F-G) Immuno-blotting showing a complete ablation of wild-type PTEN protein in PTEN mutant clones from WIBR1 (F) and WIBR2 (G) hESCs.

H-K) Analysis of BrainSpan gene expression data of PTEN (H), PAX6 (I), TBR2 (J) and DCX (K) shows PTEN expression is low in the VZ/SVZ of the human cortex at 15 or 16 post conception weeks, and high in the cortical plate (CP), a spatial pattern similar to DCX and inversely correlated with PAX6 and TBR2. IZ, intermediate zone; SP, subplate zone, MZ, marginal zone.

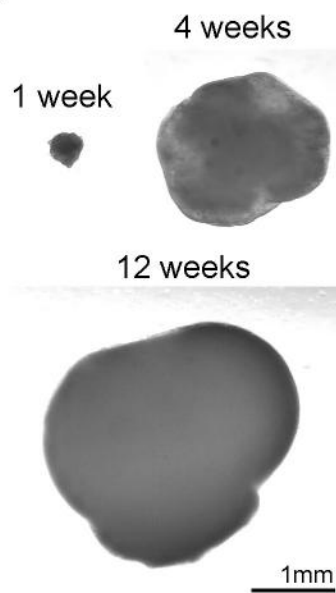
L-M) Quantitative RT-PCR analyses of PTEN in NPs, 2-week and 4-week neurons generated from WIBR1, WIBR2 and WIBR3 hESCs (L), and in WIBR3 cerebral organoids at 2, 4 and 6 weeks of age (M).

N-O) ATP assay on 2D adherent NP culture, showing enhanced proliferation in WIBR3 PTEN mutants in the presence of low PDGF (N) and IGF2 (O) concentration.

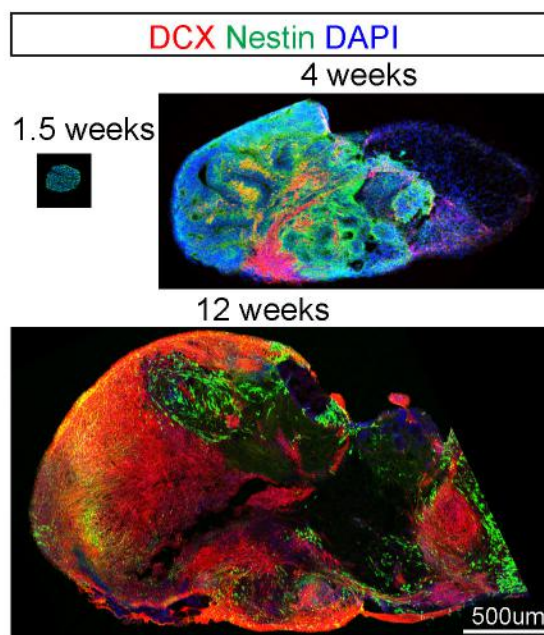
Results are mean \pm SEM. * $p < 0.05$, *** $p < 0.001$.

Figure S2

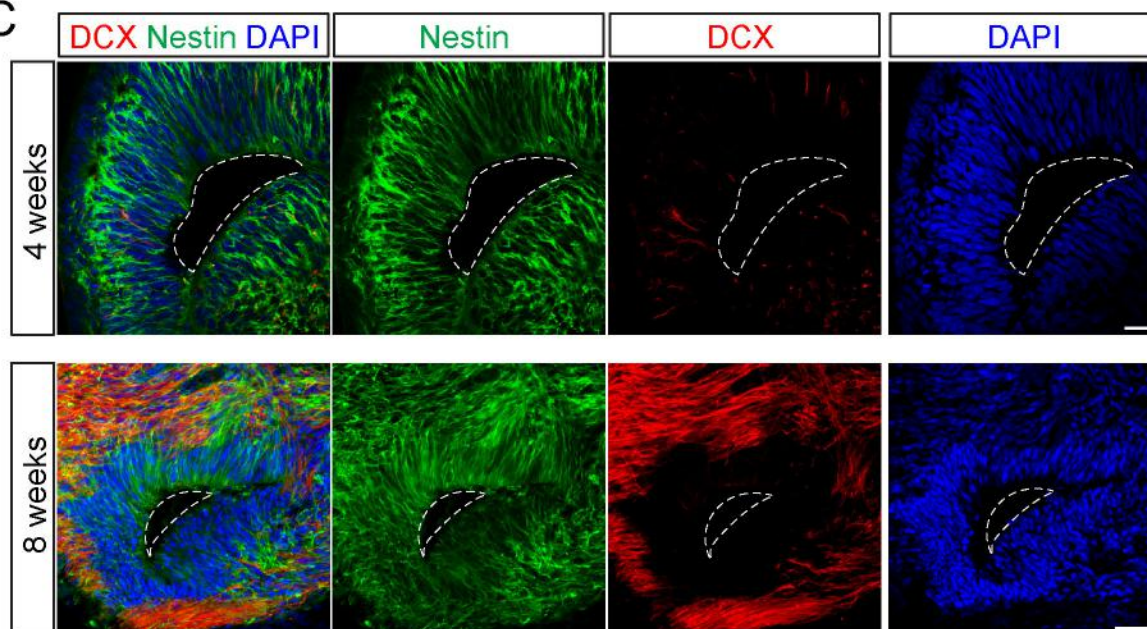
A



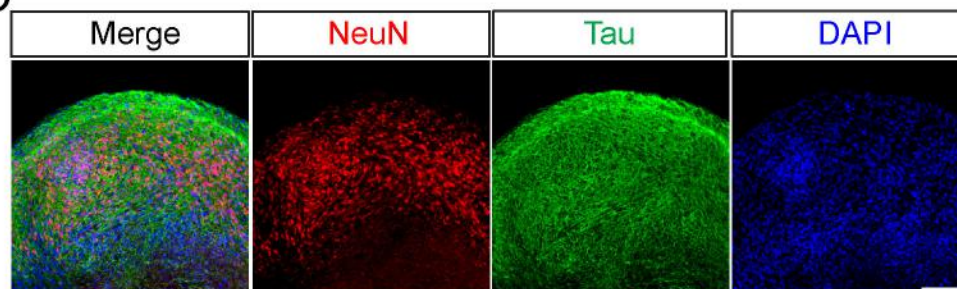
B



C



D



E

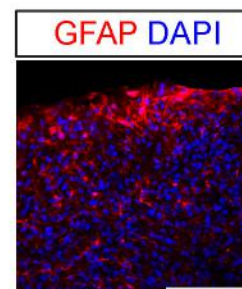


Figure S2. Generation of human cerebral organoid culture. Related to Figure 1.

A) Representative images of human cerebral organoids from wild-type WIBR3 hESCs at 1, 4 and 12 weeks. Scale bar, 1mm.

B-C) Immuno-staining with antibodies against DCX (immature neurons, red) and Nestin (NPs, green), on human cerebral organoids from wild-type WIBR3 hESCs at 1.5, 4 and 12 weeks. Low magnification images (B) demonstrate the global transition from NPs to neurons. High magnification images (C) show representative ventricle-like structures at 4 and 8 weeks, highlighting the appearance of DCX-positive immature neurons at the latter stage, and the basal location of these cells. Scale bars, 500um (B) and 50um (C).

D-E) Immuno-staining in 16-weeks-old cerebral organoids generated from wild-type WIBR3 hESCs showing abundant expression of markers for mature neurons such as NeuN and Tau (D) and astrocytes (GFAP, E). Scale bars, 100um.

Figure S3

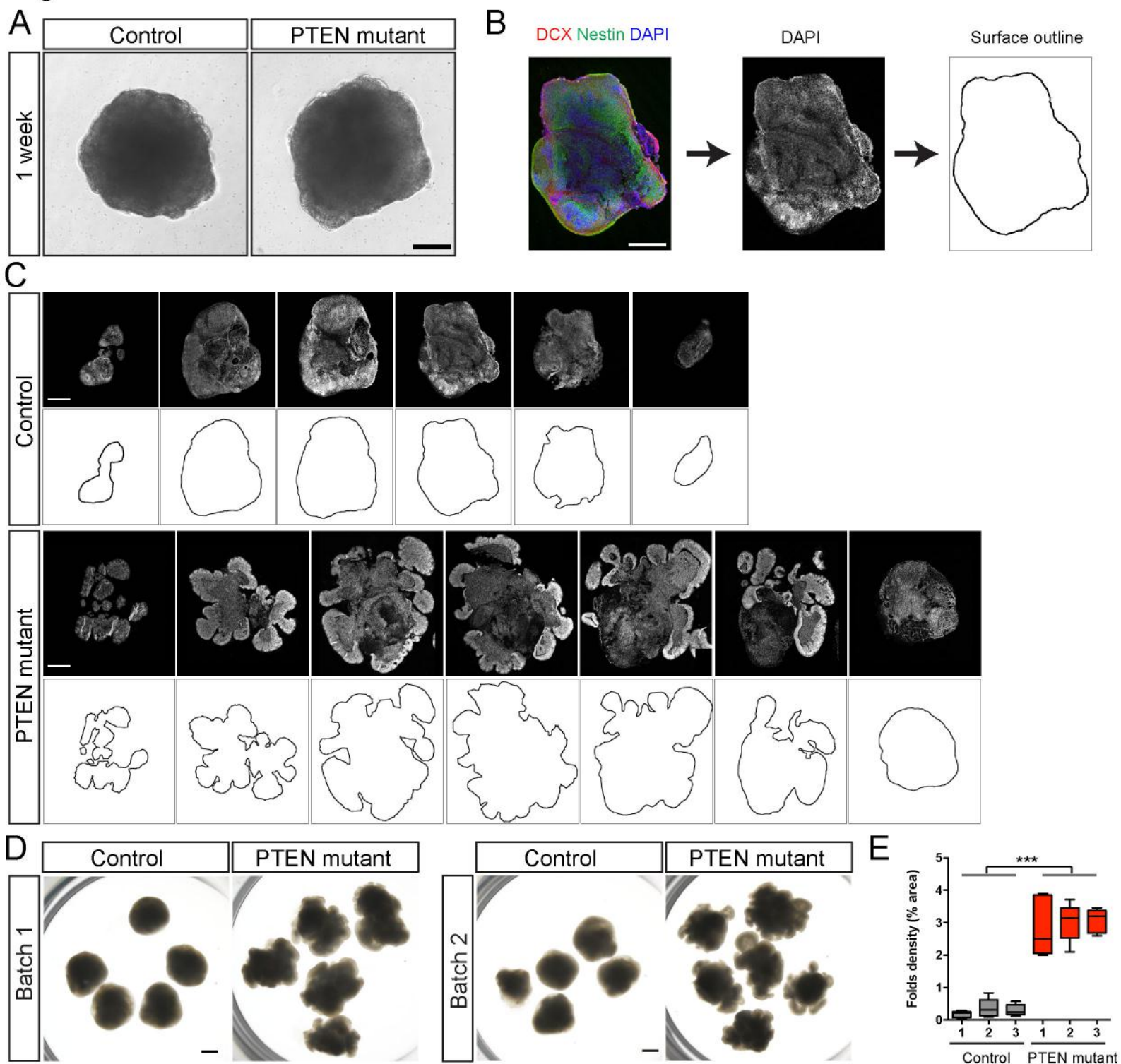


Figure S3. Morphological analysis of expansion and folding in PTEN mutant human cerebral organoids. Related to Figure 1.

A) Representative images of EBs generated from control and PTEN mutant WIBR3 hESCs at 1 week, showing their similar size and shape at this stage. Scale bar, 200um.

B-C) Images of serial histological sections from 6-week-old control and PTEN mutant WIBR3 cerebral organoids were converted into outlines for the quantification of total volume, and surface area. Scale bars, 100um.

D) Images of multiple WIBR3 organoids generated from two independent experiments showing morphological differences between control and PTEN mutants at 6 weeks of age. Scale bars, 1mm.

E) Quantification of surface folds density in Hoechst-stained control and PTEN mutant WIBR3 cerebral organoids from 3 independent experiments.

Results are mean +/- SEM. *** $p < 0.001$.

Figure S4

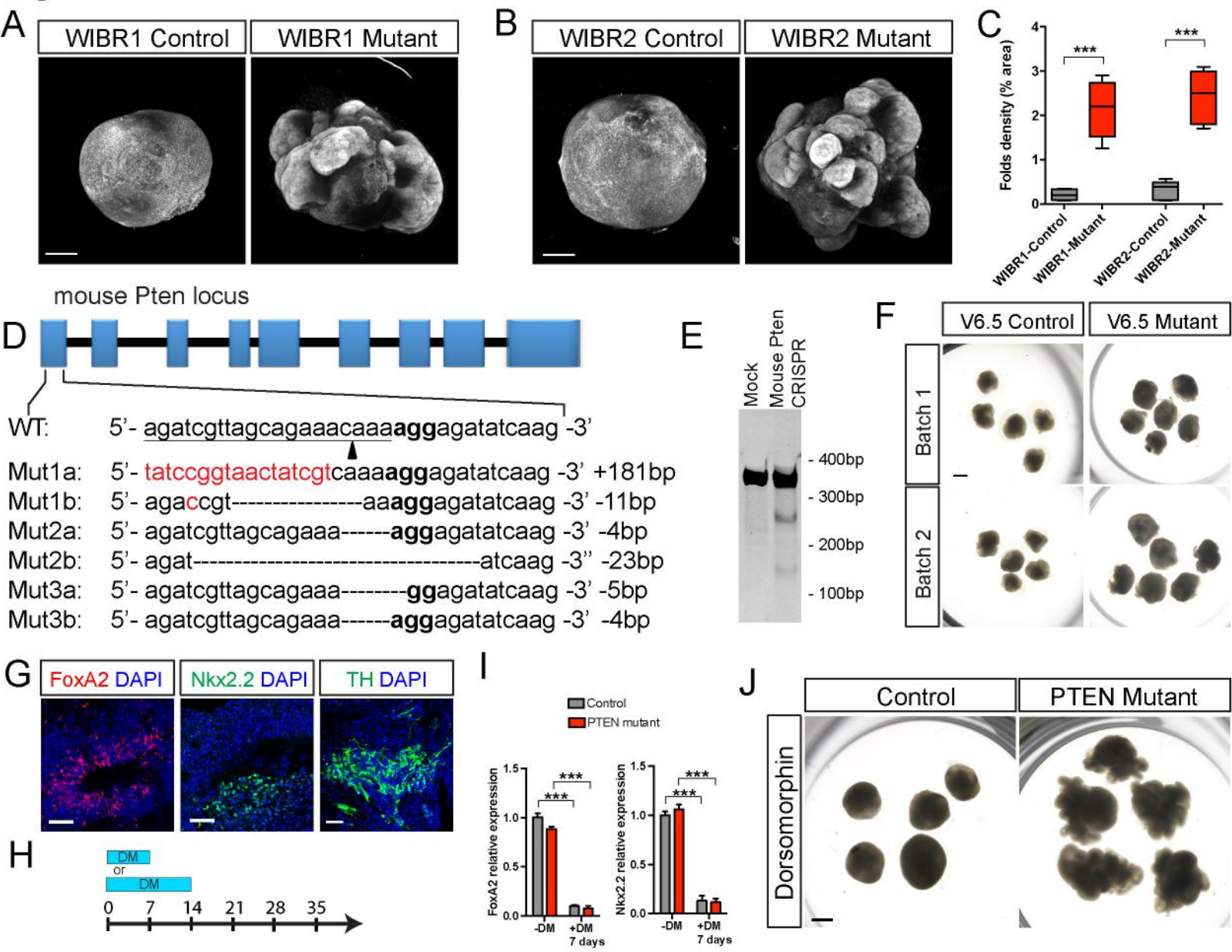


Figure S4. PTEN mutant human and mouse cerebral organoids. Related to Figure 1, 2 and 3.

A-B) Light sheet images of Hoechst-stained control and PTEN mutant human cerebral organoids generated from WIBR1 (A) and WIBR2 (B) hESCs. Scale bar, 500um.

C) Quantification of surface folds density in Hoechst-stained control and PTEN mutant human cerebral organoids from WIBR1 and WIBR2.

D) Schematic overview of the mouse *Pten* locus and sequences of mutant V6.5 mouse ESC clones,

E) Gel picture of Cel-1 assay with gRNA against mouse *Pten* gene.

F) Images of multiple mouse control and *Pten* mutant organoids at 6 weeks of age, generated from two independent experiments. Scale bars, 1mm.

G) Immuno-staining with antibodies against FoxA2, Nkx2.2 and TH on human cerebral organoids from wild-type WIBR3 hESCs.

H) Schematic diagram of dorsomorphin treatment during the first 7 or 14 days of organoid culture.

I) Quantitative RT-PCR for FoxA2 and Nkx2.2 shows dorsomorphin treatment for 7 days suppressed the expression of these none forebrain markers.

J) Images of multiple control and PTEN mutant WIBR3 cerebral organoids treated with dorsomorphin for the first 14 days. Scale bars, 1mm.

Results are mean +/- SEM. *** $p < 0.001$.

Figure S5

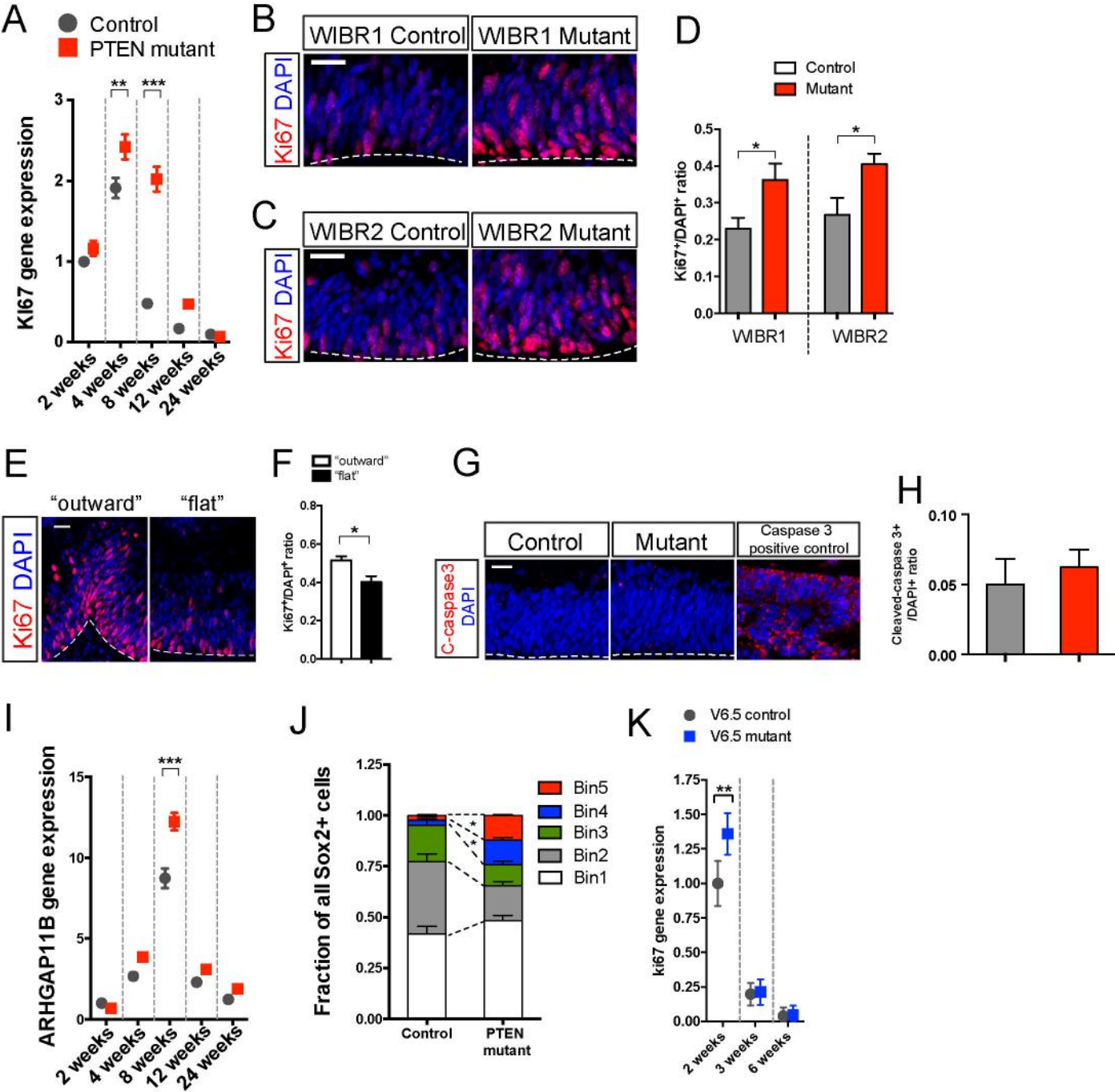


Figure S5. Enhances proliferation in PTEN mutant human cerebral organoids expand the NP pool. Related to Figure 4.

A) Quantitative RT-PCR analysis of proliferation marker KI67 demonstrates their temporal-specific over-expression in PTEN mutant WIBR3 cerebral organoids compared to controls.

B-D) Representative images and quantification of Ki67 immuno-staining in control and PTEN mutant WIBR3 cerebral organoids generated from WIBR1 and WIBR2. Scale bar, 20um.

E-F) Representative images and quantification of Ki67 immuno-staining in PTEN mutant WIBR3 cerebral organoids with outward curvature or flat. Scale bar, 20um.

G-H) Representative images and quantification of cleaved-caspase 3 (c-caspase 3) immuno-staining in control and PTEN mutant WIBR3 cerebral organoids. Positive control is a day 34 organoid after 4 days of ZIKV exposure (see later, Figure 7). Scale bar, 20um.

I) Quantitative RT-PCR analyses of ARHGAP11B in control and PTEN mutant WIBR3 cerebral organoids.

J) Distribution of Sox2+ NPs in the neuroepithelium of PTEN mutant WIBR3 cerebral organoids is significantly enriched towards the basal surface, compared to controls.

K) Quantitative RT-PCR for KI67 in mouse organoids shows mutants had increased expression at 2 weeks but not 3 or 6 weeks.

Results are mean +/- SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S6

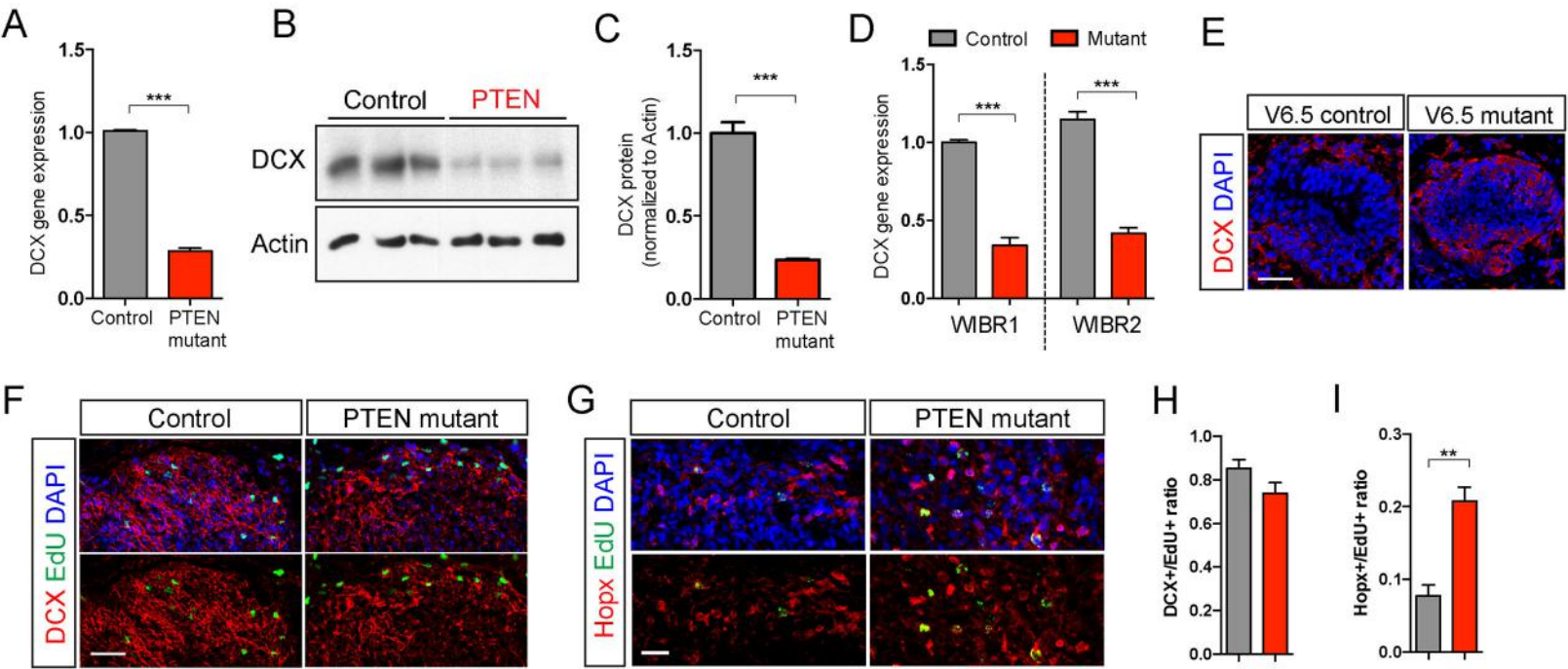


Figure S6. Transiently delayed neuronal differentiation in PTEN mutant human cerebral organoids. Related to Figure 5.

A-C) PTEN mutant cerebral organoids at 4 weeks showed delayed neuronal differentiation, as measured by reduced DCX mRNA (A) and protein (B-C) levels.

D) Delayed neuronal differentiation in 4-week-old PTEN mutant cerebral organoids from WIBR1 and WIBR2 as measured by DCX gene expression.

E) Immuno-staining for DCX in 3-week-old control and Pten mutant mouse organoids shows comparable levels of expression.

F-I) Representative images and quantification of immuno-staining for EdU (labeled at 4 weeks) and DCX (F) or Hopx (G) in 8-week old organoids. Majority of EdU+ cells co-localized with DCX (H), albeit a significantly more co-labeled with Hopx in the PTEN mutant WIBR3 cerebral organoids compared to control (I). Scale bars, 50um (F) and 20um (G).

Results are mean +/- SEM. **p<0.01, ***p<0.001.

Figure S7

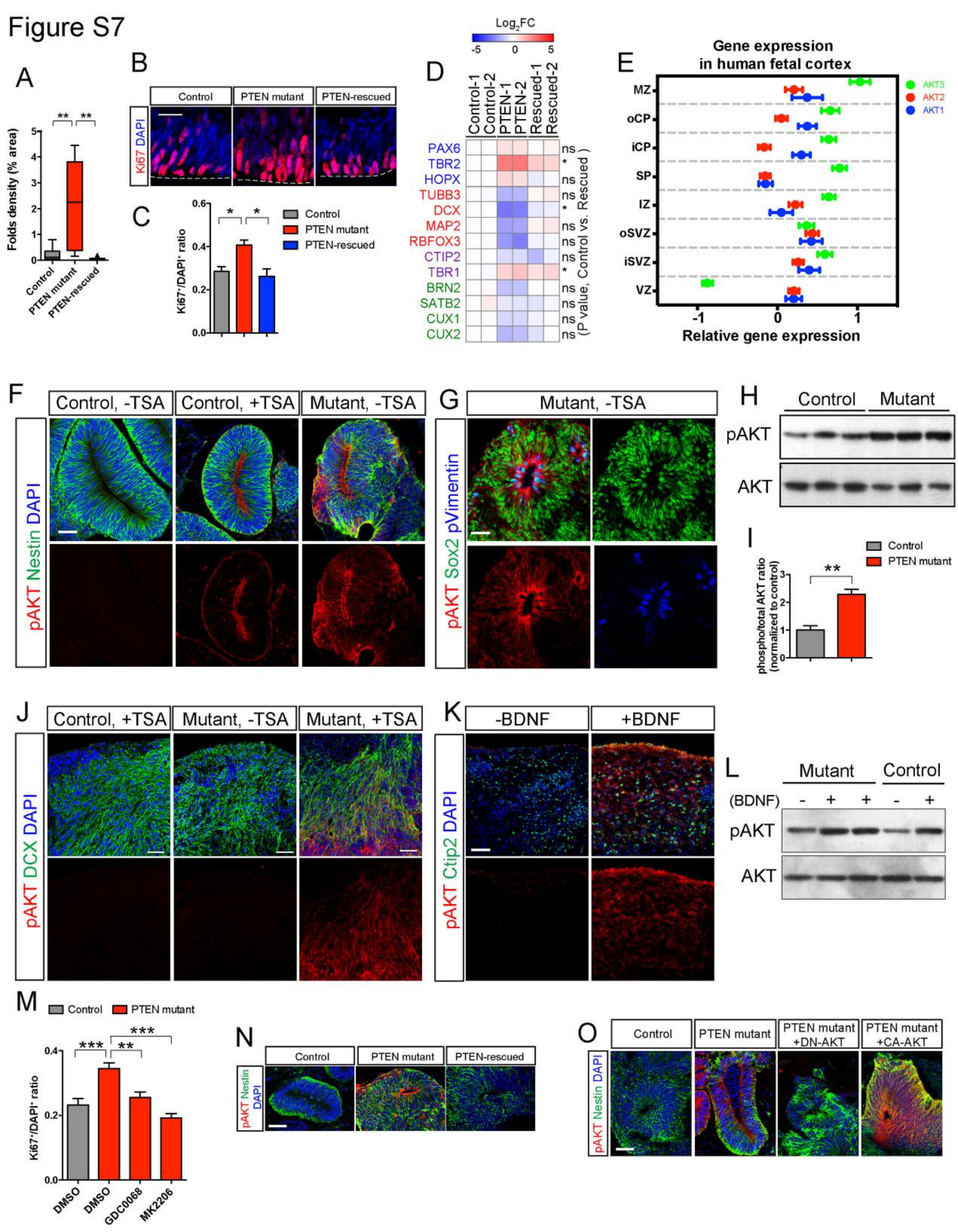


Figure S7. PTEN-AKT signaling in human cerebral organoids. Related to Figure 6.

A) Quantitative analysis of the density of surface folds in control, PTEN mutant, and PTEN-rescued WIBR3 cerebral organoids at 6 weeks.

B-C) Representative images and quantification of Ki67 immuno-staining in control, PTEN mutant, and PTEN-rescued WIBR3 cerebral organoids at 6 weeks. Scale bar, 20um.

D) Differential gene expression analyses by quantitative RT-PCR on control, PTEN mutant, and PTEN-rescued WIBR3 cerebral organoids at 6 weeks. Gene expressions are normalized to control-1. P value reflects controls vs. PTEN-rescued mutants.

E) Analysis of BrainSpan gene expression data shows all three AKT genes (1-3) are expressed in the human cortex at 15 or 16 pcw.

F) Immuno-staining with tyramide signal amplification (TSA) allowed detection of pAKT in control WIBR3 cerebral organoids, showing enrichment towards the apical surface of the VZ, a similar pattern as seen in PTEN mutants without TSA. Scale bar, 50um.

G) Immuno-staining in PTEN mutant WIBR3 cerebral organoids shows co-localization of pAKT and NP markers Sox2 and phospho-Vimentin (pVimentin). Scale bar, 50um.

H-I) Immuno-blotting analysis shows increased pAKT protein level in PTEN mutant WIBR3 cerebral organoids at 6 weeks.

J) Immuno-staining with TSA allowed detection of pAKT in DCX+ neurons of 8-week-old PTEN mutant WIBR3 cerebral organoids, no significant signal was detected in controls with or without TSA. Scale bars 50um.

K) Immuno-staining for pAKT and Ctip2 shows acute treatment with BDNF (30') increased pAKT immuno-staining in 12-week-old PTEN mutant WIBR3 cerebral organoids, without TSA. Scale bar, 50um.

L) Immuno-blotting analysis shows increased pAKT protein level upon BDNF treatment in 12-week-old control and mutant organoids.

M) Quantification of Ki67 immuno-staining shows AKT inhibitors GDC-0068 (1uM) or MK-2206 (100nM) reduced proliferation in PTEN mutant WIBR3 cerebral organoids.

N-O) Cerebral organoids generated from PTEN mutants transduced with GFP-PTEN lentivirus (N) or DN-AKT (O) showed reduced pAKT immuno-staining compared PTEN

mutants alone, whereas CA-AKT transduced mutants showed high level of pAKT. Scale bars, 50um.

Results are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S1. Antibody information. Related to Figure 1-7.

S1a. Antibodies for immuno-staining

Antibody	Vendor	Catalog #	Species	Dilution
Phospho-AKT	Cell Signaling	4058	Rabbit	1:100
Brn2	Santa Cruz	sc6029	Goat	1:500
Cleaved-caspase 3	Cell Signaling	9661	Rabbit	1:1000
Ctip2	Abcam	ab18465	Rat	1:500
Doublecortin	Santa Cruz	sc-8066	Goat	1:500
Flavivirus	Santa Cruz	sc-71122	Mouse	1:100
FoxA2	R&D Systems	AF2400	Goat	1:300
FoxG1	Abcam	ab18259	Rabbit	1:500
GFAP	Dako	z0334	Rabbit	1:1000
Phospho-Histone H3	Millipore	06-570	Rabbit	1:1000
HOPX	Santa Cruz	sc-30216	Rabbit	1:500
Ki67	Dako	M7240	Mouse	1:100
Nkx2.2	Santa Cruz	sc-15015	Goat	1:500
Nestin	Millipore	MAB5326	Mouse	1:300
NeuN	Millipore	MAB377	Mouse	1:300
Pax6	Covance	PRB-278P	Rabbit	1:300
Satb2	Abcam	ab51502	Mouse	1:300
Sox2	R&D Systems	AF2018	Goat	1:300
Tau	Dako	A0024	Rabbit	1:500
Tbr1	Abcam	ab31940	Rabbit	1:500
Tbr2	Abcam	ab23345	Rabbit	1:300
Tbr2	Millipore	AB15894	Chicken	1:300
TH	Pel-freez	P40101	Rabbit	1:500
Phospho-Vimentin	MBL	D076-3	Mouse	1:1000

S1b. Antibodies for immuno-blotting

Antibody	Vendor	Catalog #	Species	Dilution
Actin	Sigma	A2228	Mouse	1:1000
Phospho-AKT	Cell Signaling	4058	Rabbit	1:1000
AKT	Cell Signaling	9272	Rabbit	1:1000
PTEN	Cell Signaling	9559	Rabbit	1:1000

Table S2. Primer information. Related to Figure 3-6.

S2a. Primers for quantitative PCR

Gene	Primer (forward)	Primer (reverse)
Arhgap11b	AGAAAAGAAGGGCGTGTACC	TTCTTCAAAGCCTTCCAGTGA
Brn2	CGGCGGATCAAACCTGGGATTT	TTGCGCTGCGATCTTGTCTAT
Ctip2	GAGTACTGCGGCAAGGTGTT	TAGTTGCACAGCTCGCACTT
Cux1	GCTCTCATCGGCCAATCACT	TCTATGGCCTGCTCCACGT
Cux2	AAGGAGATCGAGTCGCAGAA	CTCCAGGATGCTCTTGATGG
Dcx	TCCCGGATGAATGGGTTC	GCGTACACAATCCCCTTGAAGTA
FoxA2	TGGGAGCGGTGAAGATGGAAGGGC AC	TCATGCCAGCGCCCACGTACGAC GAC
Gapdh	CGTGGAAGGACTCATGACCA	CAGTCTTCTGGGTGGCAGTGA
Hopx	GAGACCCAGGGTAGTGATTTGA	AAAAGTAATCGAAAGCCAAGCAC
Map2	CTCAGCACCGCTAACAGAGG	CATTGGCGCTTCGGACAAG
Mki67 (human)	ACGCCTGGTTACTATCAAAAGG	CAGACCCATTTACTTGTGTTGGA
Mki67 (mouse)	ATCATTGACCGCTCCTTTAGGT	GCTCGCCTTGATGGTTCCT
Nkx2.2	TGCCTCTCCTTCTGAACCTTGG	GCGAAATCTGCCACCAGTTG
Pax6	ACCCATTATCCAGATGTGTTTGCCC GAG	ATGGTGAAGCTGGGCATAGGCGG CAG
Pten	TGGATTGCGACTTAGACTTGACCT	TGGCGGTGTCATAATGTCTTTC
Rbfox3	CCAAGCGGCTACACGTCTC	CGTCCCATTGAGCTTCTCCC
Satb2	CCTCCTCCGACTGAAGACAG	TGGTCTGGGTACAGGCCTAC
Tbr1	ATGGGCAGATGGTGGTTTTA	GACGGCGATGAACTGAGTCT
Tbr2	CACCGCCACCAAACCTGAGAT	CGAACACATTGTAGTGGGCAG
Tubb3	GGCCAAGGGTCACTACACG	GCAGTCGCAGTTTTCACACTC

S2b. Primers for genotyping PCR

Gene	Primer (forward)	Primer (reverse)
Pten (human genomic)	AGCAGCTTCTGCCATCTCTC	TAGCCCTCAGGAAGAGACCA
Pten (mouse genomic)	GAGCCATTTCCATCCTGCAG	CTAGCCGAACACTCCCTAGG